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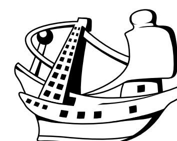

Background and induced levels of DNA damage in Pacific deep-sea vent polychaetes: the case for avoidance

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Introduction

Despite deep-sea vents being in anthropogenic terms one of the most contaminated environments on the face of the planet, relatively few studies have addressed the question of how the indigenous fauna copes with exposure to reactive oxygen species, heavy metals (e.g. mercury and cadmium) and radionuclides (e.g. radon), substances with well-documented capabilities to damage DNA, the genetic material (Dixon & Wilson, 2000; Pruski & Dixon, 2001). Consequently, our understanding of whether survival at the vents depends on resistance, enhanced DNA repair capability, or some other mechanism, currently remains unclear. While some early biochemical evidence (based on tubeworms and clams) points to the involvement of protective enzymes such as superoxide dismutase (SOD), recent work in our laboratory has suggested that the vent mussel *Bathymodiolus* may normally express higher background levels of DNA damage in its cells than is typical for non-vent species, e.g. *Mytilus* (Dixon, Wilson and Dixon, 2000). However, given the current collection methods, there is always the possibility that the marked pressure change experienced during collection (in this particular case equivalent to 175 and 240 bar) had a detrimental effect on the cellular machinery, leading to an artificially enhanced incidence of DNA strand breaks (Pruski & Dixon, submitted).

Previously, we showed for a range of alvinellid polychaetes that their rDNA melting temperature correlated positively with the thermal/chemical conditions under which they lived (Dixon et al., 1992). This we interpreted to mean that the rDNA of those species living under more extreme conditions had a higher G-C content, a phenomenon that acts to stabilize the DNA molecule (Darnell et al., 1986; Bernardi & Bernardi, 1990). Alvinellids are closely related to, or perhaps even derived from, the shallow-water ampharetids (Desbruyères & Laubier, 1986). In a related study we showed that the G-C characteristics of the rDNA of the ampharetid *Melinna palmata* Grube, which tolerates highly-reduced and metal-rich sediments (Gibbs et al., 1981), was similar to that of some alvinellids. Hence, a character that probably arose as an adaptation to life in shallow-water sediments, may have facilitated colonization of the chemically hostile hydrothermal vent environment by a common ancestor (Tunnicliffe, 1992).

In the present study we set out to investigate the effects of a mutagenic agent, hydrogen peroxide, on the DNA of two contrasting Pacific vent polychaete species, *Hesiolyra bergi* Blake, 1985 and *Paralvinella grasslei* Desbruyères & Laubier, 1982. Hydrogen peroxide was used as a proxy reactive oxygen species (ROS), which are produced during many cellular processes, including sulphide oxidation, and as a result of heavy metal exposure due to Fenton-type reactions (Phillips & Rainbow, 1994). Inhabiting different parts of the hydrothermal vent environment, these species were expected to experience contrasting metal, radionuclide and ROS exposures. Apart from their role in DNA damage, ROS are also thought to play a major part in cellular ageing (e.g. Lenaz et al., 1999). To inhibit the damaging effects of ROS, animals in general have evolved a powerful suite of antioxidants (SOD, catalase and glutathione), and if these fail there is the potential for back up from processes like DNA repair, which was also investigated during this study.

Material and methods

During the French HOPE99 cruise to active vent sites at 9° and 13°N of the East Pacific Rise, collections were made of the alvinellid *Paralvinella grasslei* (Polychaeta, Terebellida) and *Hesiolyra bergi* (Polychaeta, Phyllodocida). On board the research ship, the animals were quickly sorted and placed into chilled sea water at 6°C. Experimental exposures commenced usually within 1h of collection in either 1 or 1.5 litre containers fashioned from screw-top, water bottles. Being non-toxic and compressible these plastic bottles made excellent vessels for the high-pressure work. The high-pressure exposures (260 bar), equivalent to their natural environmental conditions, were carried out in the *Ipocamp* pressure vessel at 15°C (Shillito et al., 2001). Duplicate experiments were carried out at 1 bar.

Worms were exposed for 6h to varying hydrogen peroxide concentrations followed by a further 12h in clean sea water to allow time for DNA repair to take place. Worms were preserved for DNA analysis at t = 0, 6 and 18h. To minimize on the potentially damaging effect of gut enzymes, only the tails, i.e. approximately the last 15 segments, were excised at the end of the exposures and preserved in BLB (5% SDS, 250 mM EDTA, 50mM Tris-HCl, pH 8). Unfortunately, when the samples had reached the laboratory after the cruise, it was discovered that in the case of *Hesiolyra*, the tissues had dissolved away due to a failure by the BLB preservative to reach the tissues through the thickened cuticle, which was all that remained intact inside the sample tubes. All attempts to extract high quality DNA from either the cuticle or the tube contents failed. It was clear that the toughened cuticle had resisted preservative penetration to the extent that cellular enzymes were able to degrade the DNA as a result of autolysis linked with cell death. In contrast, in the case of *Paralvinella*, which came from a less extreme part of the vent environment, the external cuticle was thin and the tissue and DNA preservation was good.

An agarose gel electrophoresis (GE) method for measuring DNA damage was used in this study. This involved extracting genomic DNA using a standard phenol-chloroform protocol, including a RNAse step (e.g. Sambrook et al., 1989), after which 1 µg of DNA per sample was run under neutral conditions in TBE buffer through a 0.45% agarose gel at 17 volts, for 12h. Unlike the comet assay (e.g. Singh et al., 1988), the GE assay is better suited to fieldwork since there is no need for elaborate manipulation (i.e. slide making and electrophoresis) prior to samples reaching the laboratory. By focusing on double strand breaks, which are potentially more damaging in terms of their effect on the cell and the individual, the neutral version of the assay avoids some of the ambiguities that exist when trying to distinguish between naturally occurring alkali-labile sites (of which there are many in these species) and mutagen inflicted damage.

All the gel lanes were analysed using a Gel Doc 2000 imaging system and Quantity One imaging software (BioRad). Intensity profiles were generated for each lane and filtered for background noise. The 'head' and 'tail' of each lane was defined as the portion of the DNA 'comet' greater or less than a *Rf* value equal to that derived from the lambda Hind-III 23.1 kb weight marker (Fig. 1). Images of the worms were captured using a Sony video printer attached to a Wild M6 stereo microscope.

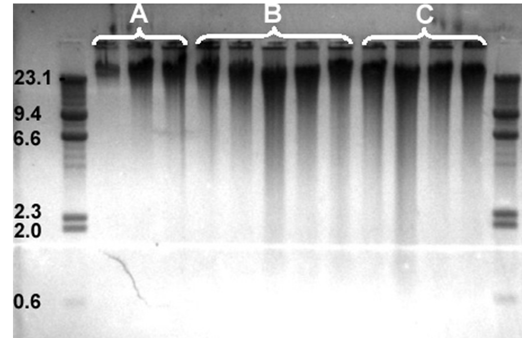

Figure 1. Representative gel showing electrophoresed genomic DNA samples from replicated worms (*Paralvinella grasslei*) exposed *in vivo* for 2.5 h at 1 bar to three different hydrogen peroxide concentrations: group A, 100 µM, Group B, 500 µM and Group C, 1 mM. The outer lanes contain lambda Hind-3 weight markers (kb). The 'comet' head refers to all the DNA located at or above the zone demarcated by the 23.1kb weight marker.

In an attempt to introduce an element of physiological reality into the experimental protocol, we carried out some exposure and recovery experiments at 260 bar, which was equivalent to the natural environmental conditions where the polychaetes lived. Unfortunately, due to health and safety considerations, it was not possible to collect animals under isobaric conditions. Hence, all were subjected to a period of depressurization during their recovery using the *Nautile* submersible. This remains a major practical shortcoming when performing any behavioural, physiological or cytological work on deep-sea species.

Comparison of means was carried out using Student's *t*-test and ANOVA, followed by Tukey tests when statistically significant differences were observed between groups.

Results

In the absence of results from *Hesiolyra bergi*, due to the reasons described above, this section deals only with the results for *Paralvinella grasslei*. Figure 1 shows a representative gel containing DNA samples from an experiment in which replicate groups of *P. grasslei* were exposed to different concentrations of hydrogen peroxide for 2.5h at 1 bar. It is evident that with increasing H_2O_2 dose there was an increase in the amount of DNA damage expressed, as visualized by an increase in tail length and fluorescence intensity (i.e. %DNA in the tail). However, there was evidence of a response plateau (or detection saturation) above 500 µM. Given the neutral pH of the gel, this DNA damage was likely to be made up largely of double-strand breaks, a class of molecular damage that leads to serious genetic and cellular effects including structural chromosomal aberrations and cell death.

Figure 2 shows results from an experiment in which *P. grasslei* were exposed to varying levels of hydrogen peroxide for 6h and then incubated in clean sea water for a further 12h to allow time for any DNA repair to take place. Duplicate experiments were carried out at 1 and 260 bar (note, only the high-pressure results are shown here). In keeping with findings for non-vent species, there was evidence of a progressive increase in DNA damage levels with increasing H_2O_2 concentration, under both pressure regimes. Tukey test results: control v. 50 µM, *P*<0.1; control v. 100µM: *P*<0.01; error bars = ±1 S.E.M. However, under neither pressure regime was there any evidence of DNA repair following removal from the H_2O_2.

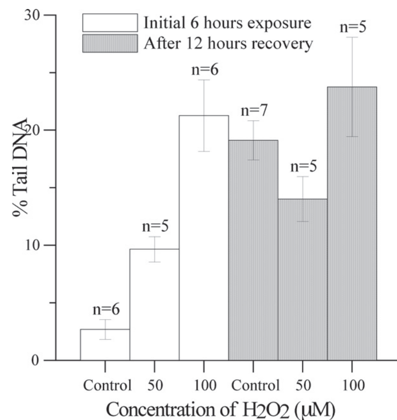

Figure 2. Results from an experiment in which *Paralvinella grasslei* were exposed to varying levels of hydrogen peroxide for 6h and then incubated in clean sea water for a further 12 h, to allow time for any DNA repair to take place. Error bars ±1 S.E.

Figure 3 shows levels of DNA damage recorded for untreated (i.e. control) *P. grasslei* after differing lengths of time at 1 and 260 bar. At 1 bar, there was no significant difference in the levels of DNA damage recorded after 6 and 18 h (Student *t*-test; *P*=0.867), whereas at 260 bar, after an initial period when the level of damage was relatively low, this rose significantly by 18h (Student *t*-test; *P*<0.001). In contrast, the physical appearance and behaviour of the animals kept at 260 bar were much healthier than those kept at 1 bar (Fig. 4). It is evident therefore, based on these DNA strand breakage results, that there was no direct correlation between an animal's external appearance and behaviour, and its internal biochemical/molecular condition. The increase in DNA damage recorded after 18 hours at 260 bar (Fig. 3) may have been a reflection of enzyme/cellular systems being better able to function at their natural ambient pressure, resulting in cellular leakage (e.g. of hydrolase enzymes), which inflicted increased levels of DNA damage (i.e. a pathological effect) not seen in animals kept at 1 bar. However, other studies have shown that animals will survive, seemingly very well, for many days under high pressure conditions in the *Ipocamp* apparatus, which suggests they may be capable of repairing much of this damage.

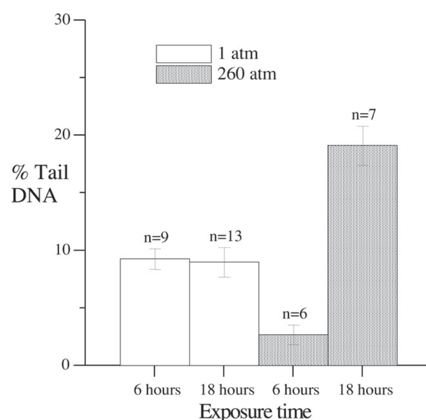

Figure 3. Levels of DNA damage recorded for untreated (i.e. control) *Paralvinella grasslei* after differing lengths of time at 1 and 260 bar. Error bars ±1 S.E.

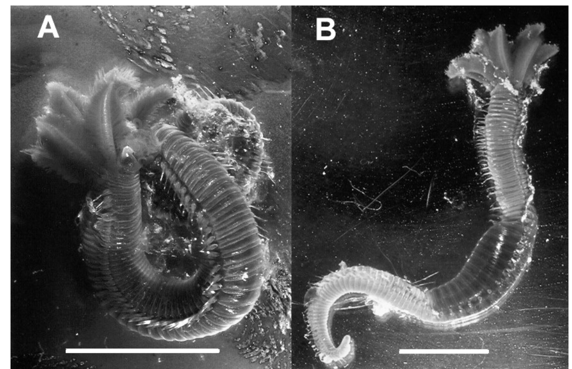

Figure 4. *Paralvinella grasslei*: **A.** An individual that was maintained at 260 bar for 18 h, exhibiting a natural healthy appearance; **B.** individual maintained at 1 bar for a similar length of time. In the latter case, there was clear evidence of physical damage (haematomas) resulting from ruptured blood vessels, probably caused by the low-pressure conditions. Paradoxically, it was the healthiest looking animals that showed the highest levels of DNA damage. Scale bar: 1 cm.

Discussion

The two worms featured in this study have contrasting lifestyles, while *Hesiolyra bergi* lives close to the hot, chemical-laden, vent emissions, *Paralvinella grasslei* is typically found associated with clumps of the giant tubeworm *Riftia pachyptila* Jones,1981 and other parts of the vent environment where the conditions are not as extreme. Given the steep chemical and thermal gradients that typify the vent habitat, these two species occupy markedly different ecological niches with regard to the potential toxicity of their environment. The environmental situation in the case of *Hesiolyra bergi,* which lives as a commensal within the tubes of the well-known Pompeii worm, *Alvinella pompejana* Desbruyères & Laubier, 1980 (Polychaeta, Terebellida), in fact turns out to be more complicated than it at first appears. While it is easy to imagine that *A. pompejana* (and hence its commensal, *H. bergi*) lives in the hottest and most chemically hostile part, recent experimental evidence (using *Ipocamp*) points to *H. bergi* having a much lower thermal tolerance (37°C

maximum: Shillito et al., 2001) than what has been claimed previously for its host (e.g. Chevaldonné et al. 1992). This inconsistency suggests that in the most extreme part of the vent metazoan biosphere, *avoidance*, through the exploitation of micro-scale distributional and behavioural elements, probably plays a more important part in vent survival than any evolutionary acquired biochemical parameters leading to increased resistance or enhanced capability to repair DNA. The results of this study were consistent with this pattern of survival.

The sensitivity of *P. grasslei* to H_2O_2 was not found to be significantly different from that recorded for the shallow-water polychaete *Pomatoceros lamarckii*, an intertidal species and filter feeder, that characteristically inhabits unpolluted conditions and which shows normal (i.e. mammalian-type) responses to mutagen exposure (Dixon et al., 1999).

In the case of a species, such as *P. grasslei*, that is a scavenger or detritivore, it is unnecessary to live in the hostile zone since particles of vent-derived organic matter are available over a wide area. Interestingly, recent laboratory maintenance experiments with *Bathymodiolus* (an endosymbiont-containing species) point to it having a lower H_2S tolerance than some earlier reports have indicated, which again points to it living under less hostile conditions (EU-VENTOX project unpublished results, 2001).

In the case of *Hesiolyra bergi,* the toughened cuticle clearly acts as an effective barrier to chemical penetration. The failure by the DNA preservative to penetrate points to this physical barrier playing an important part in the survival of a species that inhabits a more hostile part of the vent environment (this probably also applies to the carapace of *Rimicaris*). It would therefore appear that vent species exploit a range of different strategies to protect them against physical and chemical damage of which toughened exoskeletons and behavioural avoidance mechanisms play an important role.

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